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TITLE: Phase II Study of HER-2/neu Intracellular Domain Peptide-Based Vaccine Administered to State IV HER2 Positive Breast Cancer Patients Receiving Trastuzumab

PRINCIPAL INVESTIGATOR: Mary L. (Nora) Disis, M.D.

CONTRACTING ORGANIZATION:
University of Washington
Seattle, WA 98195

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14. ABSTRACT
The primary purpose of this grant is to determine the relapse free survival benefit with locally advanced and stage IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab. The scope of work includes a Phase II single arm study of a HER2 ICD peptide-based vaccine given concurrently with trastuzumab. Thirty-two patients have been enrolled during the last reporting period. All adverse events reported for these fourteen subjects are of low grade. The study has been monitored by the Fred Hutchinson Cancer Research Center without remarkable findings. Patients have developed significant T cell immune responses to the vaccine as well as epitope spreading indicating a Type I immune response is being elicited. Planned interim analysis half way through the trial suggests clinical benefit of the approach.

15. SUBJECT TERMS
Phase II, Clinical Trial, HER2, Trastuzumab

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INTRODUCTION

The scope of this study includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Enrolled patients include either: (1) patients with locally advanced HER2-positive breast cancer (Stage IIIB and IIIC) who are in complete remission and within 1 year of diagnosis and initiating treatment with chemotherapy and trastuzumab or (2) Stage IV HER2-postive breast cancer patients who are in their first complete remission and defined as NED (no evidence of disease) or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The primary objective is to estimate relapse free survival compared to a historical control of patients treated with chemotherapy and trastuzumab (44% at 4 years). We hypothesize that the relapse free survival rate at 4 years with vaccination, if successful, would be 65%. Fifty-two patients will provide 92% power to detect a statistically significant increased survival rate compared to the fixed historical rate of 44% at the one-sided significance level of p=0.05.

Secondary objectives include the assessment of the toxicity of the combined approach as well as the immunogenicity of HER2 ICD peptide vaccination. If there is evidence to suggest that the true rate of Grade IV toxicity exceeds 5% or the true rate of Grade III-IV toxicity exceeds 10% then the trial will be stopped for safety concerns. Immunogenicity of the approach will be evaluated as the ability of the vaccine to elicit HER2 ICD specific T cell immunity, to elicit epitope spreading, and to stimulate both a CD4+ and CD8+ immune response. Immune response and epitope spreading will then be modeled as time-dependent covariates in Cox proportional hazards regression models for overall survival (OS) to assess the correlation of each of these outcomes with relapse.

BODY

Task 1*: To assess the potential clinical impact of the administration of a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving concurrent trastuzumab monotherapy*

a. Construct and vial the HER2 ICD peptide vaccine. This task has been completed. The vaccine product (lot 6002) continues to be monitored at specific intervals for product stability. A Stability Study Log for lot 6002 is maintained. The study log lists the testing dates and provides a summary table to record data for each time point tested. All reserved stability vials are stored under the same conditions as the final product, -20 ± 2°C. At each stability time point reserved vials are removed from storage and visually inspected for appearance. MALDI-TOF mass spectrometry and High Performance Liquid Chromatography (HPLC) are used to confirm the stability.

Testing is performed regularly; Table 1 provides a list of test times and outcomes. In the last three tests we have observed dimerization of this vaccine. We have developed an ELISPOT assay to assess the ability of our stored vaccine to stimulate peptide specific T cell immune responses. In the assay, we use four concentrations of ICD vaccine and peptide mixture (0.1, 1, 10 and 20ug/ml) respectively to stimulate T cell responses in donors. According to the data from 10 day ELISPOT assay, the stored ICD peptide based vaccine exhibited similar ability to elicit peptide specific T cell responses *in vitro* as compared to recently constructed and purified peptides. This assay serves as a functional validation of the continuing immunogenicity of the stored vaccine.

Table 1: Product Stability Testing Results

Testing Days	Stability Conditions Met?	Dimerization	Dimerization Outcomes
90	YES	None	Not applicable
180	YES	None	Not applicable
270	YES	None	Not applicable
360	YES	None	Not applicable
540	YES	None	Not applicable
720	YES	9%	Still able to elicit peptide specific T cell responses as compared to recently constructed and purified peptides.
1080	YES	14.8%	Still able to elicit peptide specific T cell responses as compared to recently constructed and purified peptides.
1440	YES	18.8%	Assay is on-going at this time
1825	YES	19.9%	Assay is on-going at this time

b. Enroll and treat patients. This study was officially approved by the US Army Medical Research and Materiel Command (USAMRMC) Human Subjects Research Review Board (HSRRB) on June 1, 2006. To date we have enrolled 32 subjects with 9 subjects being enrolled in the last reporting period (April 27, 2009 – April 26, 2010). Table 2 demonstrates the study status of all enrolled subjects through April 26, 2010.

Table 2. Study Enrollment Table

Study Time Point	Number of subjects completed to specified time point	Off Study
Vaccine 1	1	1[a]
Vaccine 2	0	0
Vaccine 3	1	1[a]
Vaccine 4	1	0
Vaccine 5	3	1[b]
Vaccine 6	1	0
FU 1 (Month 7)	3	1[a]
FU 2 (Month 10)	3	1[a]
FU 3 (Month 14)	9	0
FU 4 (Month 18)	3	0
LTFU	7	0
Total 3	**2**	**5**

[a] Disease progression

[b] MUGA scan performed by the subject's oncology showed an ejection fraction decrease; subject also developed pneumonia and it was agreed the subject would not return to Seattle for 6th vaccine.

The literature shows that Stage IIIC and Stage IIIB patients are similar in terms of treatment (both groups receive both neoadjuvant and adjuvant chemotherapy in combination with trastuzumab for up to 12 months) and RFS and OS is similar in both groups. Therefore, we received approval as of August 29, 2008 to include Stage IIIB and Stage IIIC.

We have enrolled 9 additional subjects during the reporting period. Table 3 summarizes the stage of disease for enrolled subjects.

Table 3: Summary of Stage of Disease of Enrolled Subjects

Stage of Breast Cancer	Total Number of Subjects
Stage IV	25
Stage IIIB	4
Stage IIIC	3

Since the end of the reporting period (April 26, 2010) we have enrolled an additional subject. In addition, we are screening 4 potential subjects.

Few modifications were made to the study. Table 4 summarizes the IRB approved modifications. Neither of these modifications put the subjects at increased risk.

Table 4: Summary of IRB Approvals during April 27, 2009 – April 26, 2010

Modification Type & Comments	Approved Date
Other 1. In order to perform leukapheresis on cancer patients at the UW Clinical Research Center they require IRB approval of the leukapheresis eligibility criteria.	September 10, 2009
Protocol 1. Changes in staff. Consent 1. Changes in staff.	October 28, 2009

c. Interim statistical analysis after 25 patients have been followed for 1 year. Thirty-two of 52 patients have been enrolled in this phase II study designed to prospectively evaluate RFS and OS in HER2+ metastatic breast cancer patients who are within 6 months of achieving a CR with first-line trastuzumab-containing salvage therapy. Historical data for this patient cohort, would suggest a 4-year (from diagnosis) RFS of 44%. A planned interim analysis of RFS in the first 25 patients who have completed vaccination and have at least a 2

year follow-up was recently conducted in 2010. Interim data obtained is indicative of an improved 4-year RFS estimate of 63% in vaccinated patients.

d. Final analysis of response. Not applicable for this reporting period.

***Task 2**: To evaluate the safety of administering a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving trastuzumab monotherapy.*

a. Evaluate immediate toxicity associated with the vaccine. We use the NCI Common Toxicity Criteria (CTC) for Adverse Events Version 3.0 to grade toxicities. We pay particular attention to local reactions associated with the injection site and systemic reactions to include but not limited to fever, malaise, myalgia, nausea and headache. Table 5 summarizes the most common adverse events experienced during the last reporting period.

Table 5. Summary of Most Common Adverse Events during April 27, 2009 – April 26, 2010

Adverse Events (AE)				
	AE		Possibly, Probably, or Definitely Related	
Most Common Adverse Events	**n**	**% of all AE**	**n**	**% of All Related AE**
Injection site reaction	45	12	45	18
Hypokalemia	10	3	10	4
Hemoglobin	7	2	7	3
Lymphopenia	6	2	6	2
Leukocytes	5	1	5	2
Hypocalcemia	5	1	5	2
Fatigue	5	1	4	1
	All AE		Possibly, Probably, or Definitely Related	
Adverse Event Grading	**n**	**%**	**n**	**%**
Grade 1	330	88	226	90
Grade 2	47	12	26	10
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Grade 5	0	0	0	0

Please note that Table 5 records the most common adverse events regardless of severity. For example, a subject may have an injection site reaction at each of the three vaccines where another may not. Each one of these injection site reactions is recorded for that one subject. We have recorded a total of 377 individual adverse events for the reporting period: April 27, 2009 to April 26, 2010.

External Monitoring

As part of our Data Safety Monitoring Plan an independent monitor, assigned by the Clinical Trials Support Office at the Fred Hutchinson Cancer Research Center (FHCRC), verifies consent documentation for all newly enrolled subjects in addition to reviewing a select amount of data collected since the previous monitoring visit for randomly selected subjects. All regulatory documentation is reviewed including all IND documentation. We were monitored twice since the last reporting period: August 20-21, 2009 and March 10-11, 2010. There were no major findings. We are due again for monitoring in August 2010.

Medical Monitor Review

According to our Data Safety Monitoring Plan (DSMP) we are scheduled to meet with our Medical Monitor and related clinical research staff members bi-annually (twice a year). Prior to each meeting the Medical Monitor, Dr. Disis and related clinical research staff members are provided with an agenda and a Safety and Performance Report which includes total enrollment, adverse event reporting for and recently approved modifications and amendments for the reporting period. Meeting minutes/data are reviewed, approved and signed by the Medical Monitor. Since the last Annual Report we have presented data to our Medical Monitor on June 22, 2009 and December 30, 2009. Our next Data Safety Monitoring Plan meeting is scheduled for June 2010.

b. Determine whether there is any cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab. When subjects are enrolled we will closely monitor and document any abnormal cardiac events observed by us at clinic visits or reported to us by the subjects or physicians. All subjects have documentation of a MUGA/ECHO scan within 6 months for eligibility assessment and if that MUGA/ECHO scan is greater than 60 days old at time of eligibility we perform a MUGA/ECHO scan at their baseline visit. A follow-up MUGA/ECHO scan is performed again at 4 months post-vaccine. Table 6 compares ejection fractions at baseline and 4 Months Post-Last Vaccination.

Table 6: Baseline and 4 Month Post-Last Vaccine EF Evaluation

Subject # (n=23)	Pre-vaccine EF	4 months post-vaccine EF
12001	68%	60-65% (Echocardiogram)
12002	61%	65%
12003	65%	52%
12004	64%	Have not received follow-up documentation. Emailed physician for copy of report, we did not get a response.
12005	59%	57.5%
12006	64%	61%
12007	60%	Subject's disease progressed prior to follow-up visit.
12008	66%	51-53%
12009	56%	45.8% [a]
12010	51-52%	Subject went off Herceptin before this visit. A MUGA was not performed by her oncologist
12011	57%	55%
12012	64%	69%
12013	69%	Off Study: Progressive Disease
12014	51%	51%
12015	66%	65%
12016	50%	46%
12017	55%	60%
12018	51%	57%
12019	68%	70.8%
12020	60%	60%: This was done as an ECHO by the subject's own physician
12021	55-65%	55-65%
12022	52%	56%
12023	51%	61%
12024	62%	Off Study: Progressive Disease
12025	57%	Not yet received
12026	59%	Not yet due
12027	64%	64%; this was performed early
12028	66%	Not yet due
12029	60-65%	Off Study: Viral infection (high vial load)
12030	59%	Not yet due
12031	69%	Not yet due
12032	57.3%	Not yet due

[a] Primary oncologist is aware of EF drop. Off study per subject and last communication with her was March 25, 2008.

Two cardiac events have been observed (reported in the previous progress report), both reported during vaccination:

1. Grade 1 palpitations – Patient reports one episode of palpitations while watching TV and resolved spontaneously after about 5 minutes. No other related symptoms were reported. No other reports of palpitations have been reported since this one episode. Last episode of palpatations was one year ago while the subject was on chemotherapy.
2. Grade 2 hypotension – After vaccination 4, which included a large blood draw, patient felt presyncopal and she had to sit down. The medics were called and she had a BP of 90/50. She received IVF in transit, in ER and also when discharged home. Patient did not lose consciousness. Resolved by following study visit.

It should be noted that both of these events were for the same subject (ID#: 12010).

c. Evaluate for any potential toxicities due to the generation of an immune response to HER2. The toxicities we would expect to see for an autoimmune response to HER2 would include: (1) skin reactions such as rashes, (2) gastrointestinal events such as severe diarrhea, (3) pulmonary events, (4) change in kidney function such as a change in creatinine or (5) cardiotoxicity. All of these toxicities are closely monitored, by a credentialed clinician such as a physician and/or physician's assistance, at each clinic visit. These toxicities are recorded and monitored by routine review of systems, clinical laboratory results, and other clinical assessment (i.e. chest x-rays, MUGAs, etc.). To date our toxicity reporting does not indicate any of our 32 subjects have developed an immune response to HER2. Specifically, the toxicities observed while may have included some of the above, the adverse events have not been unexpected (e.g. skin reactions are mostly injection site reactions) and/or sustained for prolonged periods of time during or post-vaccination.

***Task 3**: To determine the immunogenicity of a HER2 ICD peptide-based vaccine in patients with Stage IV breast cancer receiving concurrent trastuzumab monotherapy*

a. Determine the immunogenicity of the approach by assessing the T cell response to HER2 ICD. To date we have evaluated the T cell responses in sixteen patients. The PBMC obtained before and after vaccination were stimulated with the three ICD peptides included in this vaccine and overlapping peptide pools for the HER2 intracellular domain (ICDpm). The T cell responses were assessed using a standard 10 day IFN-gamma(g) ELISPOT assay, the same method we reported last year. In brief, the cells were stimulated with p776, p927 p1166 and ICDpm on Day 1, and re-stimulated on Day 8. The spots of IFN-g secreted after the stimulations were counted on day 10 using an ELISPOT plate reader. Our results show that after the vaccination, the p776 specific response (HER2 specific cells/10^6 PBMN) increased 4.1 fold (pre vs. post: 99 ± 32 vs. 408 ± 96; mean ± SE; n=16. p=0.005), the p927 specific response increased 3.4 fold (pre vs. post: 162 ± 55 vs. 542 ± 134; p=0.013), and the p1166 response increased 3.5 fold (pre vs. post 182 ± 51 vs. 640 ± 130; p=0.003) after the vaccination (Fig. 1A). These patients also developed enhanced responses to ICDpm (pre vs. post: 473 ± 189 vs. 705 ± 136; n=16; p=0.328). In contrast, the response to tetanus toxoid (TT) did not obviously increase post vaccination (pre vs. post: 891 ± 147 vs. 1293 ± 200; p=0.116) (Fig. 1B). Among the sixteen patients, fourteen (88%) developed immunity to p776, twelve (75%) developed immunity to p927, thirteen (81%) developed immunity to p1166, and ten (69%) developed immunity to ICDpm (Figure 3).

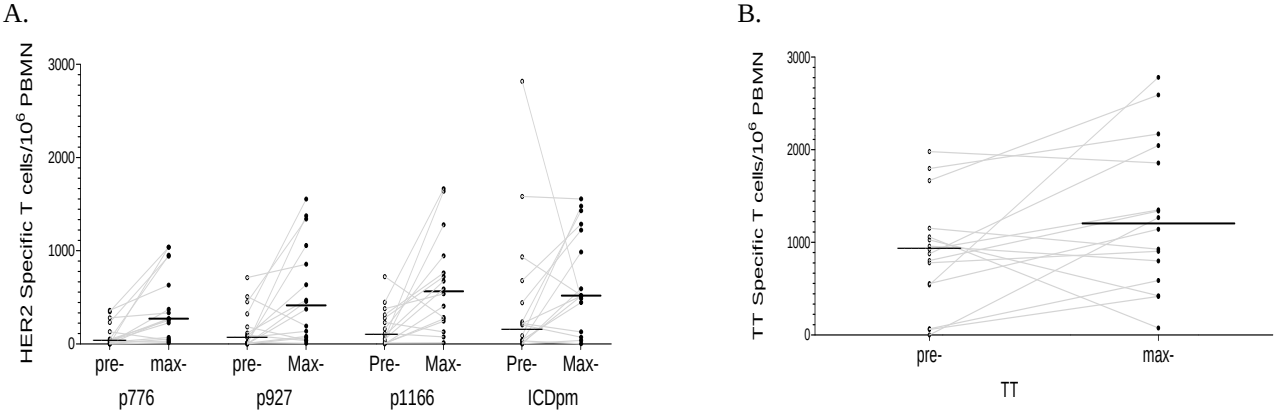

Figure 1. HER2 ICD peptide immunity elicited after the HER2 vaccination. A. HER2 antigen stimulated responses; B. TT antigen stimulated response. The bars indicate the median.

b. Determine the incidence of epitope spreading to the HER2 ICD or other peptides in the immunizing mix (intermolecular epitope spreading). We have evaluated the T cell responses to overlapping peptide pools for the HER2 extracellular domain (ECD pm), which is not included in the vaccine. The immune responses generated against HER2 ECDpm represent epitope spreading. We found that patients developed IFNg-secreting Th1 responses to ECDpm (pre vs. post: 409 ± 142 vs. 811 ± 172; p=0.082) (Fig. 2). Among the sixteen patients, twelve (75%) developed epitope spreading (Fig. 3). Our group has demonstrated that the patient's survival was significantly associated with the development of epitope spreading following vaccination (Salazar L 2009). These data suggest that these patients may have improved survival benefit after the vaccination.

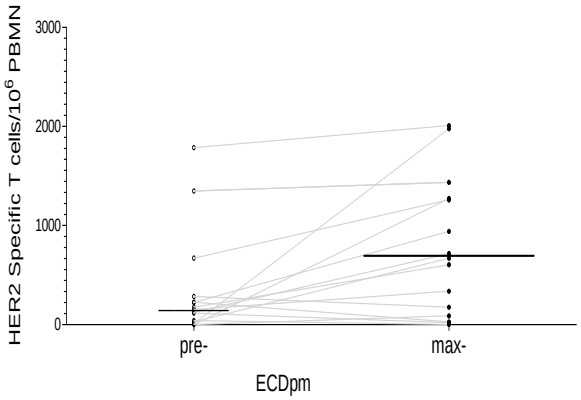

Figure 2. HER2 ECD immunity elicited after ICD peptide vaccination. The bars indicate the median.

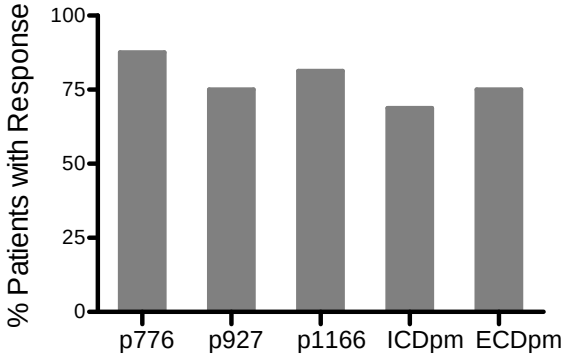

Figure 3. HER2 ICD peptide vaccine stimulated HER2 specific immunity in the majority of the patients.

Assess the serum level of TGFb. We continually evaluated the serum levels of TGF-beta (b) in patients before and after the vaccination using a human TGFb1 ELISA kit (eBioscience, San Diego, CA). TGF-b is an immunosuppressive cytokine secreted by tumor and immunosuppressive cells. We found that the levels of serum TGFb decreased in 11 of the 18 patients evaluated after the 3rd vaccination. The mean level of hTGFb was 1460 (± 37) pg/ml before the vaccination. It decreased to 984 (± 30) pg/ml after the 3rd vaccine, and maintained at 985 (± 25) pg/ml after 6th vaccine (mean ± SE, n=18; Fig. 4). Thus, the mean level of serum TGFb decreased more than 33% after vaccination, although it did not reach statistical significance. The decreased levels of serum TGFb may predict a better prognosis as elevated levels of serum TGFb are associated with an increased risk of relapse in breast cancer patients (Bates GJ et al J Clin Oncol 2006).

Correlate the serum TGFb with HER2 specific IFNg-secreting Th1 response. We analyzed the correlation between the change of serum levels of TGFb post vaccination and HER2 ICD vaccine-induced T cell response at the same time. We found that the greater the magnitude of HER2 specific T cell response, as demonstrated by IFNg secretion, the greater the decrease in serum TGFb. The increased T cell response to ICDpm correlated with decreased levels of TGFb (p=0.097, r=0.445, Fig 5A). The correlation between increased epitope spreading T cell response and decreased levels of TGFb was significant (p=0.0397, r=0.535, Fig 5B). In contrast, there was no correlation between the magnitude of TT response and the change in TGF-b levels (p=0.889; r=0.041, Fig 5C). Thus, the increased numbers of HER2 antigen, especially HER2 ECD, elicited via HER ICD peptide vaccination was associated with a decrease in serum TGF-b levels.

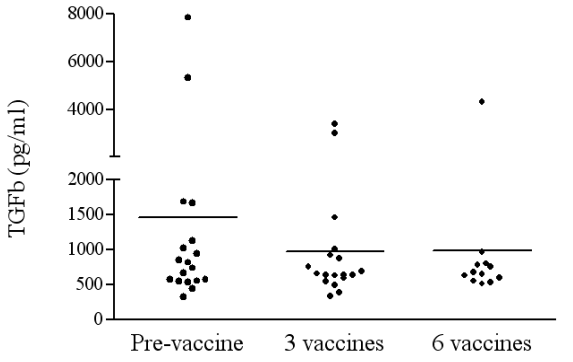

Figure 4. Levels of TGFb decreased in the serum of patients after vaccination with HER2 ICD peptides. The bars indicate the mean.

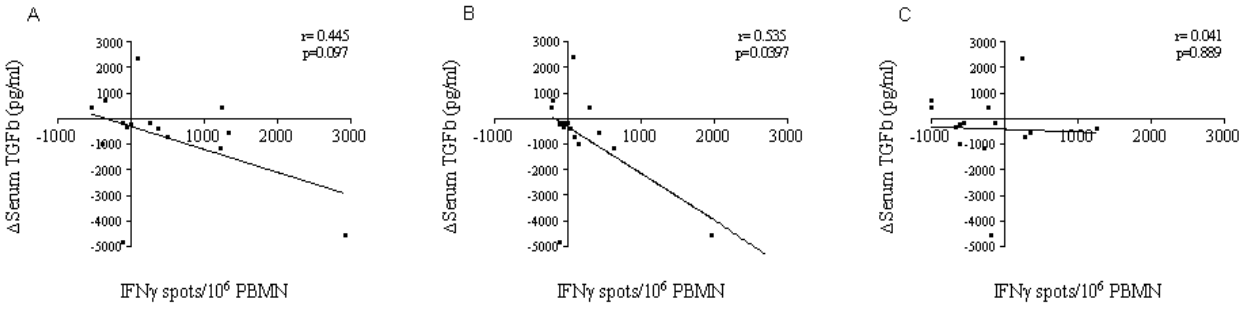

Figure 5. HER2 ICD peptide vaccine induced ICD peptide response (A) and epitope spreading (B), not tetanus toxoid response (C), after immunization were associated with a decrease in serum TGF-beta levels. X axis: IFNg secretion (post-pre vaccine); Y axis: serum levels of TGFb (Post-pre vaccine).

Evaluate multiple CD4 T cell cytokine responses induced by the vaccine. CD4+ T cell mediated immune responses to tumor antigens elicited after vaccinations are central to the efficacy of tumor vaccine. Multiple tumor specific Th1 (IFNg/TNFa/IL2), or Th1/Th17 cells may be superior to IFNg-secreting Th1 cells alone in the prevention of tumor relapse. Th17 is a subset of CD4 Th cytokine recently reported and has been found to have anti-tumor effects in tumor-bearing animals. We assessed the levels of multiple cytokines secreted from PBMN collected 1 month after the 3rd vaccine with those from pre-vaccinated PBMN as control. The supernatants collected on Day 8 after the antigen stimulation from the 10 day IFNg ELISPOT assays as described above were used for this assay. Simultaneous detection of multiple cytokines (IFN-γ, TNF-α, IL-1b, IL-4, IL-5, IL-10, and IL-17) was performed using a custom multiplex cytokine kit (Millipore) on Luminex. Figure 6 shows preliminary data from eight patients. Data collected via cytokine multiplexing is color coded as to the magnitude of antigen specific cytokine increase (red) or decrease (blue) with vaccination. Both ICDpm and ECDpm induced responses are shown. The data suggest several specific patterns of Th response to the HER2 antigens. Half of the patients increased IFNg/TNFa responses (Patients 11, 12, 14 and 17); half of the patients increased IFNg/IL17 and IFNg/Th17/Th2 responses (patient 9, 11, 12 and 17). In contrast, Patient 16's T cells decreased both Th1 and Th17 cytokine production after stimulation. We will further determine the levels of multiple cytokine secretion after the vaccination and evaluate their significance in the prevention of tumor relapse.

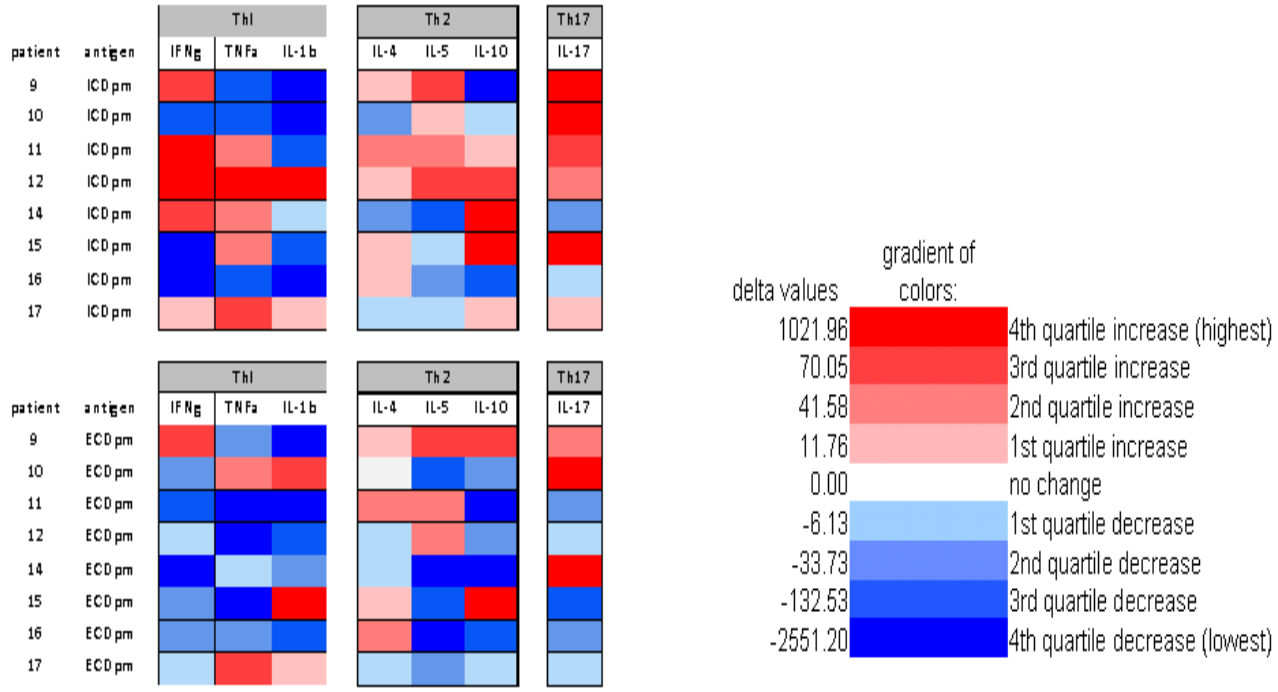

Figure 6. Cytokine secretion patterns induced by HER2 vaccination. Data is expressed as post-vaccine minus pre-vaccine cytokine level. Dark red: 4th (highest) quartile increase in cytokine level with descending red colors reflecting the 3rd, 2nd, and 1st quartile increase respectively. Dark blue: 4th (highest) quartile decrease in cytokine level with descending blue colors reflecting the 3rd, 2nd, and 1st quartile decrease respectively.

c. Determine the incidence of epitope spreading to other immunogenic proteins associated with breast cancers (extramolecular epitope spreading). Not applicable to this reporting period.

d. Assess the absolute magnitude of the CD4+ and CD8+ HER2 specific immune responses generated after active immunization. Not applicable to this reporting period.

e. Evaluate the generation of HER2 specific antibody immunity and antibody avidity. We have not been able to develop a HER2 antibody assays that would allow reproducible identification of HER2 specific antibody responses while patients are receiving trastuzumab (as trastuzumab interferes with the assay). These experiments will be considered on hold.

f. Determine whether overall survival is associated with the development of HER2 specific T cell response or epitope spreading after active immunization. Not applicable to this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

- Increased enrollment
- Interim analysis on first 25 subjects
- Demonstrated significant augmentation of immunity in vaccinated patients
- Demonstrated epitope spreading in the majority of patients evaluated to date

REPORTABLE OUTCOMES: none at this time

CONCLUSIONS

We began study enrollment on December 29, 2006. We have since enrolled 32 subjects who are at varying phases of vaccination and follow-up. To date we have observed only low grade adverse events (Grades 1 & 2) most of which were expected.

Although we are performing our immunologic analysis as patients have completed early studies suggest we are both significantly augmenting a vaccinated immune response as well as generating epitope spreading in the majority of patients.

In order to successfully accomplish the scope of work for this project, we have requested a no cost extension to continue enrollment to the study. Once this funding has expired we will be using another funding source in order to meet the final accrual to this study of 52 subjects. We intend to complete accrual by December 2010.